Intracellular pH is a promising biomarker of early neurodegeneration, as shown by ^{31}P MRS in a 3-NP rodent model of Huntington's disease

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Introduction

Huntington's disease (HD) is a neurodegenerative disorder characterized by abnormal movements (chorea), dementia, and preferential degeneration of the striatum (1). Even if mechanisms of neurodegeneration in HD remain partially unknown, it had been shown that mitochondrial defects - in particular anomalies in succinate dehydrogenase SDH activity - might play a key role in the pathogenesis of this disorder (2-4). In this context, the attempt to find MR-detectable biomarkers reflecting metabolic anomalies is still needed (5-7). In this study, we propose to investigate the potential of pH measurement by ^{31}P spectroscopy as a relevant early biomarker of neurodegeneration in a rodent model of HD.

Materials & Methods

The main goal of this study being to detect cerebral pH variations before lesions outbreak, a well-characterized model of chronic intoxication using 3-nitropropionic acid (3-NP) was chosen (8). One of the main characteristics of this model being a brutal onset of striatal lesions 5 to 6 days after the beginning of intoxication, time was left to measure cerebral pH prior lesions outcome.

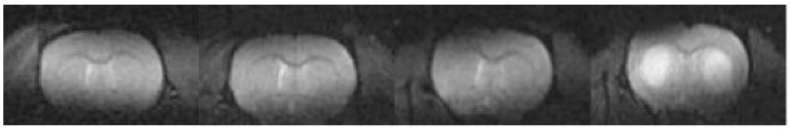

Figure 1 – 2D RARE images during the intoxication protocol These images were acquired on the same animal. An axial slice crossing the striata is presented, showing that (i) no lesion is visible until D3 included and (ii) major striatal lesions (T2 hypersignal) are detected at D5.

NMR system All NMR experiments were performed on a 4T magnet (Magnex, Abingdon, UK), equipped with high performance gradients (20cm ID, 200 mT.m^{-1}, 260µs rise time) and interfaced to an Avance (Bruker, Ettlingen, Germany) console. A custom-built ^{31}P RF surface coil (∅2.5cm, f_0=68.97MHz) was used to measure brain pH. Shimming procedure and anatomical imaging were performed using a ^{1}H surface coil (∅5cm, f_0=170.28MHz, Bruker, Ettlingen, Germany).

Animals and intoxication protocol The study was conducted on 5 male Lewis rats (336±9g, 10-week-old, Iffa Credo, L'Arbresle, France). A solution of 3-NP was delivered by chronic infusion (54 mg.kg^{-1}.d^{-1}) using osmotic minipumps (flow rate 10 µl/hr, model 2ML1; Alzet, Palo Alto, CA) implanted subcutaneously in the back of the animals under ketamine–xylazine anesthesia (8).

Animal handling For NMR experiments, animals were secured in the prone position in a custom-built cradle. A water-heating pad was used to keep the body temperature stable. Animal anesthesia was induced with 5% isoflurane in a 1.5 ml.min^{-1} oxygen flow and maintained with 2% isoflurane in a 1.5 ml.min^{-1} oxygen flow applied with a face mask allowing free breathing.

MR acquisitions A T_2-weighted imaging sequence was optimised to detect cerebral lesions (RARE, TE/TR=60/3000ms, 192×192 matrix, 300µm in-plane resolution). pH measurements were performed in a 10×7×8mm^3 VOI using an optimized PRESS sequence (TE/TR=8.1/4000ms, 1024points, 5900Hz spectral width). Each animal underwent MR sessions (including imaging+pH measurement) at 4 different stages of the disease: before pump implantation (**Ctrl**), 1 day after (**D1**), 3 days after (**D3**) and 5 days after (**D5**).

Determination of cerebral pH For each stage (Ctrl, D1, D3 and D5), ^{31}P spectra were summed over the five animals. Summed spectra were quantified using an original basis set describing 13 ^{31}P multiplets, implemented for AMARES method in jMRUI (9, 10). pH was calculated by the software from the chemical shift of Pi relative to PCr measured on ^{31}P spectra (δ, see Fig. 1) (11). The parameters of Pi-PCr system were set to pK=6.77, δ_{HA}=3.23ppm and δ_A=5.70ppm.

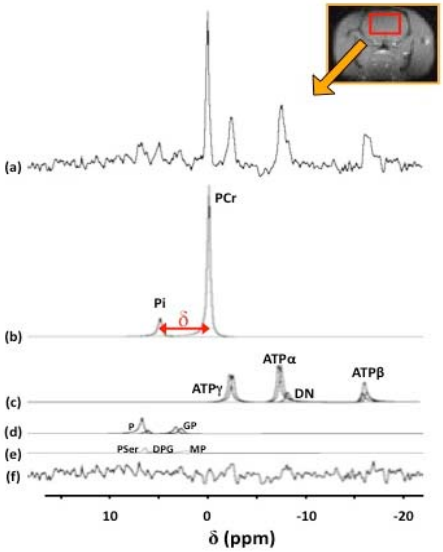

Figure 2 – AMARES quantification (a) PRESS spectrum summed over the 5 rats (D1, lb=35Hz). Contributions of (b) Pi and PCr, (c) ATP and DN, (d) P (PE and PC) and GP (GPE and GPC), (e) PSer, DPG and MP. (f) Residuals.

Results and discussion

Animal behavior Symptoms of drowsiness, slowness of movement appeared at D3 for all 3-NP-treated rats. At D5, all the rats had developed severe and permanent dystonia of hindlimbs (12-14).

Detection of striatal lesions Fig. 1 shows the 4 anatomic MR images acquired on the same animal (Ctrl, D1, D3 and D5). No major lesion was visible until D5. In contrast, massive striatal lesions were detected at D5 on the five animals, as shown by strong T2 hypersignal in both striata.

Measurement of cerebral pH AMARES analysis of one ^{31}P spectrum summed over the 5 animals is presented in Fig. 2. Results are shown in Fig. 3. A significant increase in cerebral pH is detected between D0 and D3 (pH_{D0}=7.08±0.03 vs. pH_{D3}=7.17±0.02), showing that pH variations occur before the onset of striatal lesions, and consequently before detectable neurodegeneration.

Using the well-characterized 3-NP model allowed us to compare changes in brain pH with changes in SDH inhibition reported on the exact same model of chronic intoxication (8). It must be kept in mind that the inhibition of the mitochondrial enzyme SDH - which has been demonstrated in animal models as well as in patients - is a key feature of HD. As shown on Fig. 3, pH changes measured in our study are highly correlated with SDH inhibition ($p<0.05$). Therefore intracellular pH appears associated with early metabolic impairment.

This study is in agreement with 2 recent reports of cerebral pH increase in HD mice (Q111 model) (15) and HD patients (16). Importantly however, these studies were post-symptomatic. As a consequence, our study is the first report of pH increase prior the onset of lesions in an animal model of HD.

In conclusion, pH changes measured by ^{31}P MRS in an animal model of HD present 2 striking features: (i) pH changes precede MRI detected degeneration, (ii) pH changes are correlated with metabolic impairment at the mitochondrial level. Therefore MRS-measured brain pH appears as a promising biomarker for HD.

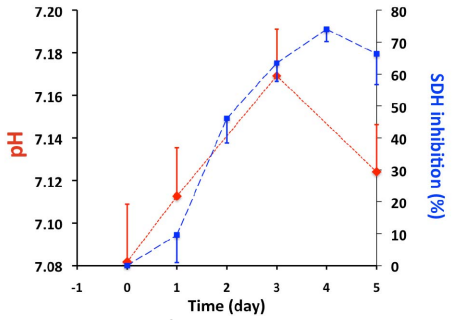

Figure 3 – Evolution of cerebral pH during the intoxication protocol A significant increase in cerebral pH (♦) is detected between D0 and D3, followed by a significant decrease on D5. pH variations appear correlated with SDH inhibition (■) (8).

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